Chapter 6 – HOW CELLS GROW

6.1 (p. 155) Introduction –

1. Substrates + Cells → Extracellular Products + More Cells (6.1)
2. $\Sigma S + X \rightarrow \Sigma P + nX$
3. **Net Specific Growth Rate**:

$$\mu_{net} = \frac{1}{X}\frac{dX}{dt} \quad (6.2a)$$

4. $\mu_{net} = u_g - k_d$ (6.2b)
5. Cell growth in terms of cell number (concentration):

$$\mu_R = \frac{1}{N}\frac{dN}{dt}, \quad \mu_R = \text{net specific replication rate} \quad (6.3)$$

μ'_R indicates that $k_d \simeq 0$ (cell death unimportant or ignored)

6.2 (p. 156) Batch Growth

6.2.1 Quantifying Cell Concentration

6.2.1.1 Determine Cell Number Density

1. Petroff-Hausser slide or hemocytometer
2. Petri dishes (agar) – viable cells – "plate count" method
3. Particle counter (Fig. 6.1)

6.2.1.2 Determine Cell Mass Concentration

1. Direct methods -
   - 1.1 Dry weight
   - 1.2 Packed cell volume (centrifuge fermentation broth)
   - 1.3 Optical density (turbidity) using spectrometer for light transmission
2. Indirect methods – fermentation process (molds) (S or P)
   - 2.1 Intracellular components (RNA, DNA, protein) (Fig. 6.2)
     - 2.1.1 DNA & protein concs. remain fairly constant
     - 2.1.2 Cell Protein – total amino acids (Biuret, Lowry, Kjeldahl)
   - 2.2 Intracellular ATP concentration (mg ATP/mg cells) ~ constant

$$\text{luciferin} + O_2 \xrightarrow[\text{luciferase}]{} \text{light} \quad (6.4)$$

ATP conc. of a typical bacterial cell = 1 mg ATP/g dry-weight cell

** 2.3 Nutrients used for cell mass production (N, P, S, O_2, carbon source)

Note: This relates to the stoichiometry material covered previously

2.4 Products of cell metabolism -

- 2.4.1 – anaerobic conditions – ethanol, lactic acid
- 2.4.2 – aerobic conditions – CO_2
- 2.4.3 – changes in pH (e.g., nitrification, denitrification)
- 2.4.4 – viscosity change – due to extracellular polysaccharide

6.2 (p. 160) Growth Patterns and Kinetics in Batch Culture

1. Batch growth curve–Figure 6.3: phases include: lag, log, deceleration, stationary, death
2. Lag – adaptation of cells to a new environment

Note: (1) Many commercial fermentation plants rely on batch culture.

(2) <u>To obtain high productivity, the lag phase must be as short as possible</u>

2.1 Low concs of some nutrients and growth factors can extend the lag phase

2.2 Example – *Enterobacter aerogenes* – lag phase increases as Mg^{2+} is decreased (Mg^{2+} is a activator of the enzyme phosphatase)

2.3 Example – heterotrophic cells require CO_2 fixation, and excessive sparging removes metabolically generated CO_2 too rapidy

2.4 Age of inoculum culture (how long maintained in a batch condition)

minimize – cells are adapted to growth medium/conditions before inoculation;

- cells should be exponential phase
- inoculum size should be large (5% to 10% by volume)

2.5 Multiple lag phases – diauxic growth (see Example 4.1)

3. Log (or exponential) phase – balanced growth – all cells grow at same rate (average composition remains constant) -

3.1 Net Specific Growth Rate remains the same during this phase

3.2 Exponential growth rate is First Order with respect to X

$$\frac{dX}{dt} = \mu_{net} X, \qquad X=X_0 \text{ at } t=0 \tag{6.5}$$

Integration yields:
$$\ln \frac{X}{X_0} = \mu_{net}\, t \quad \text{or } X=X_0 e^{u(net)t} \tag{6.6}$$

Where X and X_0 are cell cons at time t and t=0.

3.3 Doubling time (time required to double microbial mass or number):

$$t_d = \frac{\ln\ 2}{\mu_{net}} = \frac{0.693}{\mu_{net}} \tag{6.7}$$

$$t'_d = \frac{\ln\ 2}{\mu'_R} \tag{6.8}$$

4. Deceleration phase -

1. Depletion of one or more essential nutrients, or
2. Accumulation of toxic by-products of growth
3. Changes occur over a very short period of time (log to deceleration phase)
4. Cell physiology under conditions of nutrient limitations are studies in continuous culture

5. Stationary phase – net growth rate = 0

1. Growth rate = death rate
2. Metabolites – primary = growth-related
   - secondary = nongrowth-related
3. Phenomena occurring during Stationary phase-
   (1) Total cell mass constant, but number of viable cells decrease
   (2) Cell lysis, mass decrease, 2nd growth on lysis products

(3) Secondary metabolites – produced as results of deregulation

4. Endogenous metabolism–catabolism-cell reserves for building blocks & energy

(1) $\frac{dX}{dt} = -k_d X$ or $X = X_{so} e^{-k(d)t}$ (6.9)

X_{so} = cell mass conc. at beginning of Stationary phase.

Because S = 0, $u_g = 0$ in Stationary phase

(2) If inhibitory product is produce and accumulates (ethanol-yeast): manage by

1). Dilution of toxified medium

2). Add unmetabolizable chemical compound to complex the toxin

3). Simultaneous removal of toxin

6. Death phase – rate of death usually follows first-order kinetics:

$$\frac{dX}{dt} = -k'_d N \quad \text{or} \quad N = N_s e^{-dk'(d)t} \qquad (6.10)$$

where N_s is the concentration of cells at the end of the Stationary phase, and k'_d is the first-order death rate constant.

7. Yield Coefficient:

Growth Yield in a fermentation is: $Y_{X/S} = -\frac{\Delta X}{\Delta S}$ (6.11)

Apparent Growth Yield at end of a fermentation (batch growth period)

$$\Delta S = \Delta S_{\text{assimilation}} + \Delta S_{\text{assimilated into}} + \Delta S_{\text{growth}} + \Delta S_{\text{maintenance}} \qquad (6.12)$$

into biomass extracell. prod. energy energy

Other yield coefficients: $Y_{X/O(2)} = -\frac{\Delta X}{\Delta O_2}$; $Y_{P/S} = -\frac{\Delta P}{\Delta S}$ (6.13, 6.14)

Table 6.1 (p. 167) lists values of $Y_{X/S}$ and $Y_{X/O(2)}$ for substrates & organisms

Maintenance Coefficient = specific rate of substrate uptake for cell maintenance:

$$M = -\frac{[dS/dt]_m}{X} \qquad (6.15)$$

(1) During Stationary phase – endogenous metabolism is used for “M”

(2) Maintenance – repair damage components, transfer nutrients in/out of cells, motility, adjust osmolarity of cell interior.

8. Microbial products are classified into three major categories (Fig. 6.6, p. 168)

1. Growth-associated products are produced simultaneously with microbial growth:

$$q_p = \frac{1}{X}\frac{dP}{dt} = Y_{P/X}\,\mu_g \qquad (6.16)$$

e.g., production of a constitutive enzyme

2. Nongrowth-associated product formation occurs during Stationary Phase

Specific rate of Product formation is constant" $q_P = \beta =$ constant (6.17)

e.g., Secondary metabolites – antibiotics (e.g., penicillin)

3. Mixed-growth-associated product formation occurs during slow growth & Stationary phases. Specific rate of product formation:

$$q_P = \alpha\mu_g + \beta \quad (6.18)$$

e.g., lactic acid fermentation, xanthan gum, & some secondary metabolites.

9. **Example 6.1 – Growth rate & Yield - Solution:**

1. Max Specific New Growth Rate: $\mu_{net} = \frac{\ln 37.5 - \ln 5.1}{t_2 - t_1} = 0.1\ h^{-1}$

2. Apparent Growth Yield: $Y = \frac{\Delta X}{\Delta S} = \frac{41 - 1.25}{0.63 - 100} = 0.4$ g cell/g subtrate

3. Maximum cell concentration if 150 g glucose used with same inoculum size

$$X_{max} = X_0 + YS_o = 1.25 + 1.4(150) = 60.25 \text{ g cells/L}$$

6.2.3 (p. 169) – Now Environmental Conditions affect Growth Kinetics

1. Temperature above optimal level – specific replication rate: Fig. 6.7

$$\frac{dN}{dt} = (\mu'_R - k'_d)\ N \quad (6.19)$$

1). Both μ'_R and k'_d vary with temperature (Arrhenius Eqn)

$$\mu'_R = Ae^{-E(a)/RT}\ ,\ k'_d = A'e^{-E(d)/RT} \quad (6.20)$$

Ea = activation energy for thermal growth (10-20 kcal/mol)

Ed = activation energy for thermal death (60-80 kcal/mol)

2). Temp – affects Yield coefficient $Y_{X/S}$ (e.g., single-cell protein production)

1). Affects Maintenance coefficient, m (E_m = 15-20 kcal/mol)

3). Temp – affects rate-limiting step in fermentation process

(1) Rate of bioreaction > rate of diffusion (immobilized cell system)

(2). Ea for molecular diff = 6 kcal/mol; Ea for bioreactions > 10 kcal/mol

2. pH – bacteria (3-8), animal cells (6.5-7.5), non-optimum => "m" increase

NO_3^- utilization causes increase in pH

Organic acid production decreases pH

CO_2 production affects pH

3. Dissolved Oxygen – consumption rate > supply rate at high cell concentration

1) Growth rate can be proportion to O_2 conc. (Michaelis-Menten relation)
2) Fig. 6.9 (p. 172) – growth rate as f(O_2), while mass is determined by S
3) Critical O_2 as % DO_{sat}: 5-10% (bacteria, yeast); 10-50% (mold)

4) Rate of O_2 transfer: $N_{O(2)}$ (mg O_2/l-hr) = $k_L a(C^* - C_L)$ = OTR (6.21)

k_L = oxygen transfer coefficient (cm/h), a = gas-liquid interfacial area (cm^2/cm^3)

$k_L a$ = volumetric oxygen transfer coefficient (h^{-1}), C^*=Sat., C_L=Do conc.

5) Oxygen Uptake Rate: OUR (mg O_2/h) = $q_{O(2)}X = \dfrac{\mu_g X}{Y_{X/O(2)}}$ (6.22)

6) $$\text{OTR} = \text{OUR}: \frac{\mu_g X}{Y_{X/O(2)}} = k_L a(C^* - C_L) \qquad (6.23)$$

or

7) $$\frac{dX}{dt} = Y_{X/O(2)}\, k_L a(C^* - C_L) \qquad (6.24)$$

p. 173 8) Redox Potential:

$$E_h = E'_o + \frac{RT}{4F} \log P_{O(2)} + 2.3 \frac{RT}{F} \log (H^+) \qquad (6.25)$$

9) Ionic Strength –

(1) affects transport of nutrients in and out of cells, and
(2) solubility of O_2
(3) $$I = \frac{1}{2} \Sigma C_i Z_i^2 \qquad (6.26)$$

e.g., NaCl inhibitory at > 40 g/L (osmotic pressure)
max conc.: glucose = 100 g/L; ethanol 50 g/L

6.2.4 Heat Generation by Microbial Growth (you read)

6.3 p. 175. Quantifying Growth Kinetics

6.3.1 Introduction

Structured model – divides cells (mass) into components

Unstructured model – assumes fixed cell (mass) composition (balanced growth)

Valid in single-stage, steady-state continuous culture & log phase of batch culture

Fails during any transient condition

Segregation – divide culture into individual units (cells)- differ from each other

6.3.2 **Unstructured Nonsegregated** Models to Predict Specific Growth Rate

6.3.2.1. Substrate-limited growth:

* p.176 $$\mu_\gamma = \frac{\mu_\mu S}{K_S + S}$$; Fig. 6.11; **MONOD EQN**. (6.30)

- Michaelis - Menten kinetics for enzymes applied to whole cells for growth
- If endogenous metabolism is not important, then $\mu_{net} = \mu_\gamma$

* p. 177 For rapidly growing, dense cultures:

$$\mu_\gamma = \frac{\mu_m S}{K_{SO} S_O + S} \qquad (6.31)$$

Where S_O is the initial substrate concentration, and K_{SO} is dimensionless

Skip equations (6.33) through (6.38)

6.3.2.2. Models with growth inhibitors: High S, P, and with inhibitory substances, growth rate depends on inhibitor concentration.

1. Substrate inhibition - skip
2. Product Inhibition –

(a) Competitive: $$\mu_\gamma = \frac{\mu_m S}{K_S [1 + P/K_P] + S} \qquad (6.42)$$

(b) Noncompetitive: $$\mu_\gamma = \frac{\mu_m}{[1 + K_S/S] [1 + P/K_P]} \qquad (6.43)$$

Example: Ethanol fermentation from glucose. ETOH is inhibitor at concentrations above 5%.

3. Inhibition by toxic compounds – same as enzyme kinetic expressions

(a) Competitive: $$\mu_g = \frac{\mu_m S}{K_S[1 + I/K_I] + S} \qquad (6.46)$$

(b) Noncompetitive: $$\mu_g = \frac{\mu_m}{[1 + K_S/S] [1+ I/K_I]} \qquad (6.47)$$

(c) Uncompetitive: $\mu_g = \dfrac{\mu_m \; S}{\left[\dfrac{K_S}{[1+I/K_I]} + S\right] [1 + I/K_I]}$ (6.48)

(d) include death term in rate expression:

$$\mu_g = \frac{\mu_m \; S}{K_S + S} - k'_d \qquad (6.49)$$

6.3.2.3 The Logistic Equation: To describe growth curve in Fig. 6.3, combine growth equation (6.2) with Monod equation (6.30) and assume no endogenous metabolism

$$\frac{dX}{dt} = \mu_{net} X \qquad (6.2a)$$

$$\mu_{net} = \frac{\mu_m \; S}{K_S + S}, \qquad (6.30)$$

To get: $$\frac{dX}{dt} = \frac{\mu_u \; S}{K_S + S} X \qquad (6.50)$$

Relationship between microbial yield (Y) and substrate consumption is:

$$Y = \frac{dX}{dS} \rightarrow dX = y \; dS$$

Integrate over X and S to get: $X - X_O = Y_{X/S} (S_O - S)$ (6.51)

See text for Equations (6.52) and (6.53)

Logistic equations are a set of equations that characterize GROWTH in terms of CARRYING CAPACITY (CC). Specific growth is related to amount of unused CC.

$$\mu_g = k [1 - X/X_{OO}], \; X_{oo} = CC \qquad (6.54)$$

Therefore: $$\mu = \frac{dX}{dt \; X} \rightarrow \frac{dX}{dt} = kX [1 - X/X_{OO}] \qquad (6.55)$$

$$X = \frac{X_O e^{kt}}{1 - \dfrac{X_O}{X_{OO}} (1-e^{kt})} \qquad (6.56)$$

Equ. (6.56) is represented by the growth curve in Fig. 6.12 (p. 182)

Study - Example 6.2 Logistic Equation, pages 181-182

6.3.2.4 p. 183. Growth models for filamentous organisms or submerged microbial pellet SKIP

6.3.3 . p.183. Models for Transient Behavior – shift in environmental or cultural conditions.

6.3.3.2. Chemically Structured Models (Fig. 6.14).

1. All reaction should be expressed in terms of intrinsic concentrations = amount of a compound per unit cell mass or cell volume. [Extrinsic concentration = amount of a compound per unit reactor volume – cannot be used in kinetic expressions.

2. The dilution of intrinsic concentration by growth must be considered.

$$\frac{d[V_R C_i]}{dt} = V_R X + r_{fi} \qquad (6.60)$$

6.3.4 p. 189. Cybernetic Models – Process is goal seeking (e.g., maximization of growth rate).

1. Initially motivated by desire to predict response of a microbial culture to growth on a set of substitutable carbon sources.
2. Recently – identify regulatory structure of a complex biochemical reaction network (e.g. **cellular metabolism**).
3. Newest use – metabolic engineering – relating information on **DNA sequences in an organism to physiologic function** (See Chapter 8).

6.4 p. 189. How Cells Grow in Continuous Culture

6.4.1 Introduction -

1. Constant environmental conditions for growth, product formation
2. Determine response of cells to the environment

6.4.2. Specific Devices for Continuous Culture-

1. Chemostat (Constant chemical environment) – constant nutrient, cell, product
2. Turbidostat – cell concentration maintained constant. Study environmental stress, select cell variants or mutants with desirable properties
3. Plug Flow Reactor (PFR)

6.4.3 Ideal Chemostat = CFSTR (with pH and DO control units) (Fig. 6.18)

Material balance on cell concentration around the chemostat:

$$FX_O - FX + [\mu_g X V_R - k_d X V_R] = \frac{dX}{dt} V_R \quad (6.64)$$

Solve for $$\frac{dX}{Dt} = \frac{FX_O}{V_R} - \frac{FX}{V_R} + \frac{[\mu_g X V_R}{V_R} - \frac{k_d X V_R]}{V_R} \quad (6.64a)$$

$\frac{F}{V}$ = D (dilution rate) = $\frac{1}{\theta}$, where θ = retention time in chemostat

From (6.64): $$FX_O - FX + [\mu_g X V_R - k_d X V_R] = \frac{dX}{dt} V_R$$

$$0 \quad - FX + [\mu_g X V_R - 0] = 0 \text{ (steady state)}$$

Therefore $$FX = \mu_g X V_R$$

Divide by X $$F = \mu_g V_R$$

Solve for μ_g $$m_{\mathbf{g}} = \frac{F}{V_R} = \frac{1}{\theta} \text{ .=. } \mathbf{D} \quad (6.66)$$

Therefore, setting the Dilution (D) rate sets the Growth Rate (μg)

Now substitute Monod equation: $$\mu_g = D = \frac{\mu_{max} \; S}{K_S + S} \quad (6.67)$$

Find μ_{max} , K_S - from plot of $1/\mu_g$ versus 1/S

$$\frac{1}{\mu_g} = \frac{K_S + S}{\mu_{max} \; S} = \frac{K_S}{\mu_{max} \; S} + \frac{S}{\mu_{max} \; S} = \frac{K_S}{\mu_{max}} \frac{1}{S} + \frac{1}{\mu_{max}}$$

$$\mathbf{Y} = \quad \mathbf{m} \quad \mathbf{X} + \mathbf{b}$$

Use (6.67) to solve for (relate) S as a function of D:

$$D (K_S + S) = \mu_{max} \; S$$

$$D K_S + DS = \mu_{max} \; S$$

$$D K_S = \mu_{max} \; S - DS = S (\mu_{max} - D)$$

Therefore $$S = \frac{D K_S}{(\mu_{max} - D)} \quad (6.68)$$

Material balance on limiting substrate around the chemostat

$$FS_O - FS - \mu_g X V_R \frac{1}{Y^M_{X/S}} - q_P X V_R \frac{1}{Y_{P/S}} = \frac{dS}{dt} V_R \qquad (6.69)$$

$$FS_O - FS - \mu_g X V_R \frac{1}{Y^M_{X/S}} - 0 \text{ (extracell prod)} = 0 \text{ (steady state)}$$

$$F(S_O - S) = \mu_g X V_R \frac{1}{Y^M_{X/S}}$$

$$\frac{F(S_O - S)}{V_R} = D(S_O - S) = \frac{\mu_g X}{Y^M_{X/S}} \qquad (6.70)$$

Since $\mu_g = D$ at steady state, if $k_d = 0$, then; $D(S_O - S) = \frac{DX}{Y^M_{X/S}}$

Therefore $$X = Y^M_{X/S}(S_O - S) \qquad (6.71)$$

Using Equation 6.68, the steady-state cell concentration can be expressed as:

$$X = Y^M_{X/S}\left(S_O - \frac{D K_S}{(\mu_{max} - D)}\right) \qquad (6.72)$$

Now lets consider endogenous metabolism:

Equ 6.66 ($D = \mu_g$) becomes $D = \mu_g - k_d = \mu_{net}$ (6.73a)

Or $\mu_g = D + k_d$ (6.73b)

Substitute (6.73b) into steady-state substrate balance, and extracell P=0, then

Eqn 6.69 becomes: $D(S_O - S) - \frac{(D + k_d)\, X}{Y^M_{X/S}} = 0$ (6.73c)

Where $Y^M_{X/S}$ denotes the maximum yield coefficient (no maintenance or endogenous respiration).

Eqn (6.73c) can be rearranged to: $D\frac{(S_O - S)}{X} - \frac{(D + k_d)}{Y^M_{X/S}} = 0$ (6.74)

$$D\left(\frac{1}{Y^{A/P}_{X/S}}\right) - \frac{D}{Y^M_{X/S}} - \frac{k_d}{Y^M_{X/S}} = 0 \quad (6.75a)$$

Divide by D and solve for $1/Y^{A/P}_{X/S}$:

$$\frac{1}{Y^{A/P}_{X/S}} = \frac{1}{Y^M_{X/S}} + \frac{k_d}{Y^M_{X/S}\, D} \quad (6.75b)$$

$$\frac{1}{Y^{A/P}_{X/S}} = \frac{1}{Y^M_{X/S}} + \frac{m_S}{D} \quad (6.76)$$

$$\mathbf{Y = b + m\ X}$$

Where $m_S = \frac{k_d}{Y^M_{X/S}}$ (6.77)

While $Y^M_{X/S}$ is a constant, $Y^{A/P}_{X/S}$ varies with growth conditions if $k_d > 0$

Find $Y^M_{X/S}$ and m_S: plot $1/ Y^{A/P}_{X/S}$ versus 1/D; slope= m_S, intercept = $1/ Y^M_{X/S}$